The battle of words in gender groups: A language-based approach to gender differences

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The B6.SW Bilineal Congenic Sucrose Octaacetate (SOA)-Taster Mice

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SWR/J inbred mice (Tasters) reliably avoid, whereas C57BL/6J inbred mice (Nontasters) are indifferent to, sucrose octaacetate (SOA) at certain concentrations. From these strains we have developed a set of bilineal congenic Taster mice. Approximately 4000 mice, from 2 isogenic and 12 segregating generations, were tested in a program designed to evaluate genetic models for SOA tasting during development of congenic strains. The criterion phenotype was avoidance or nonavoidance in preference tests of the bitter tastant SOA at concentrations of 10^{-4} and 10^{-5} M. Across the 12 segregating generations, the results were consistent with Mendelian expectations for a single autosomal locus with complete dominance of the Taster phenotype. The breeding program produced 12 replicate B6.SW lines containing the taster allele on the B6-Nontaster genomic background. The congenic Taster mice may facilitate a functional analysis of the sense of taste.

KEY WORDS: single locus; sucrose octaacetate; taste genetics; congenic lines; mouse; *Mus domesticus*.

INTRODUCTION

Substantial evidence has accumulated that when testing is conducted at appropriate concentrations, the avoidance of sucrose octaacetate (SOA) in two-bottle preference tests segregates according to expectations from a single-locus autosomal Mendelian model with complete dominance of the Taster (strong avoidance) phenotype (Warren and Lewis, 1970; Lush, 1981; Whitney and Harder, 1986; Gannon and Whitney 1989). The results

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from testing various inbred strains with behavioral psychophysical procedures (Harder *et al.*, 1984) and peripheral neural electrophysiological procedures (Shingai and Beidler, 1985) are consistent with the possibility that the segregating SOA avoidance phenotype is mediated by a peripheral mechanism which influences taste sensitivity. Here we report the results of congenic line development based on the behavioral phenotype, avoidance of the bitter tastant SOA.

A congenic line is developed through a successive series of selective lineal backcrosses, in which one strain provides a genetic background (called a first parent or inbred partner) and another strain provides a specific chromosome segment containing a differential locus (called a second parent or donor strain). The resulting new congenic strain is, in theory, genetically identical to the first parent except for a small chromosome segment surrounding the differential locus which has been transferred from the donor strain. The variant of the basic breeding design used for the development of congenic lines in a particular case depends on the mode of inheritance of the target phenotype, specifically on dominance (ability to identify heterozygotes) (Flaherty, 1981). When both the inbred partner and the second parent are inbred strains, a resultant congenic line may be referred to as a bilineal congenic strain. For further analyses of phenotypes a replicated set of bilineal congenic strains combines many of the advantages of congenic lines with those of recombinant inbred strains (Bailey, 1981). The present attempt to develop congenic Taster lines was undertaken for two reasons. One was that results accumulated across segregating generations during their develpment could serve to test the hypothesis of single-locus influence on the SOA avoidance phenotype. The second reason was that, if successful, the resulting bilineal congenic Taster lines could provide the basis for a fundamental analysis of the sense of taste.

METHOD

Subjects

The subjects were inbred and hybrid mice (*Mus domesticus*) from C57BL/6J (first parent; B6) and SWR/J (donor strain; SW) inbred-strain mice. Progenitor inbreds and periodic replacement B6 mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. Details of husbandry are provided elsewhere (Whitney and Harder, 1986).

Apparatus and Test Procedures

Individually caged mice, at least 40 days of age, were subjected to two-bottle preference tests, with SOA (Sigma Chemical Co., St. Louis,

Mo.) dissolved in distilled water presented in one bottle versus distilled water alone presented in the other bottle. Most individuals were tested for two 24-h periods with a concentration of 10^{-5} *M* SOA vs. distilled water and then tested for another two 24-h periods with 10^{-4} *M* SOA vs. distilled water. For generation N_2 testing was conducted only at 10^{-5} *M* SOA, and at N_3 some individuals were tested only at 10^{-5} *M* SOA. The 10^{-4} *M* test was reinstituted during generation N_3 because concurrent research with other genotypes suggested that 10^{-5} and 10^{-4} *M* did not always yield identical results (Harder and Whitney, 1985; Gannon and Whitney, 1989). The number of individuals for each generation that completed testing at each concentration is given in Table I. For each concentration the position of the bottles was interchanged after the first 24-h period to control for position effects. An individual subject's score for a concentration was the average of the two daily preference ratios (PR)

Table I. Observed Ratios of Taster (T)[a] to Nontaster (N)[b] SWR/J and C57BL/6J Inbred-Strain Mice, Their F_1 and F_2 Progeny, Ten Lineal Backcross Generations, and a Subsequent Intercross Generation, in Sucrose Octaacetate (SOA) Preference Tests at Two Concentrations: For the Segregating Generations, Observed Ratios Are Compared to Ratios Expected Under One-Locus, Two-Locus Additive, and Two-Locus Epistatic Models

	No. tested		Observed %				Expected %		
			10^{-5} *M* SOA		10^{-4} *M* SOA				
Generation	10^{-5} *M*	10^{-4} *M*	T	N	T	N	One locus, T:N	Two additive loci, T:N	Two epistatic loci, T:N
SWR/J	405	387	98.0	2.0	100.0	.0			
C57BL/6J	371	363	.8	99.2	.3	99.7			
F_1 (N_1)	141	126	97.9	2.1	100.0	.0			
F_2	481	483	72.8	27.2	74.8	25.2	75:25	56:44**	94:6**
N_2	84	—	58.3	41.7	—	—	50:50	25:75*	75:25*
N_3	107	57	40.2	59.8	52.6	47.4	50:50	25:75**	58:42*
N_4	227	227	55.1	44.9	55.5	44.5	50:50	25:75**	54:46
N_5	211	212	53.6	46.4	52.1	47.9	50:50	25:75**	52:48
N_6	187	186	55.6	44.4	55.4	44.6	50:50	25:75**	51:49
N_7	192	189	53.1	46.9	52.4	47.6	50:50	25:75**	50:50
N_8	229	229	42.4	57.6	43.9	56.1	50:50	25:75**	50:50
N_9	211	210	48.8	51.2	49.3	50.7	50:50	25:75**	50:50
N_{10}	304	306	48.7	51.3	49.8	50.2	50:50	25:75**	50:50
N_{11}	359	358	51.3	48.7	51.3	48.7	50:50	25:75**	50:50
$N_{11}F_1$	489	485	75.9	24.1	75.7	24.3	75:25	56:44**	75:25

[a] Preference ratio $<.15$.
[b] Preference ratio $\geq.15$.
* $p < .001$ at 10^{-5} *M* SOA only.
** $p < .001$ at 10^{-5} and 10^{-4} *M* SOA.

at that concentration (PR = amount SOA solution consumed/total amount consumed from both bottles). Further details are given by Whitney and Harder (1986). Across the hybrid generations, about 20 to 40 individuals were tested simultaneously in a particular experimental "run." In each of the resulting ~140 experimental runs, a few (typically two to four) individuals from both the SW and the B6 inbred lines were also included as bioassay procedural controls.

Selection and Breeding Procedures

Based on previous results (Harder *et al.*, 1984; Whitney and Harder, 1986), the phenotypic selection criterion for an individual Taster mouse (SW-like) was a preference ratio $<.1$ at both 10^{-5} and 10^{-4} *M* SOA (except where only 10^{-5} *M* SOA was tested). Individuals with a preference ratio $\geq .1$ were classified as Nontasters (B6-like). F_1 and F_2 generations were bred from reciprocal crosses of the B6 and SW mice. Ten lineal backcross generations (N_2–N_{11}) were then bred by mating a phenotypic Taster hybrid mouse to a B6 inbred partner mouse. The lineal backcrossing began at generation N_2, with all four reciprocal $F_1 \times$ B6 matings equally represented among 12 mating pairs. As diagrammed in Fig. 1, this is the backcross or NX breeding system with 12 replicates, appropriate for congenic strain development when the differential allele from the donor strain is autosomal dominant (Flaherty, 1981).

The inbred partner mice were bred and raised in our laboratory except that periodically C57BL/6J mice from Jackson Laboratories served as inbred partners. This use of B6 mice from Jackson Laboratories was to assure that the genetic background of the eventual congenic mice would remain as close as practicable to that of the standard C57BL/6J inbred strain. All F_1, F_2, and backcross progeny in the first two surviving litters from each mating pair were tested. Occasionally further individuals were also tested, for instance, when a Taster of a particular sex was desired for continuation of a particular backcross line. Taster individuals from generation N_{11} (presumptive heterozygotes at the differential locus) were sib mated to initiate the new congenic strains.

RESULTS AND DISCUSSION

Cumulated across 4 years of testing, many more data are now available from the progenitor inbred strains than were reported previously (Whitney and Harder, 1986). Figure 2 illustrates preference ratios of more than 700 SWR/J and C57BL/6J inbred mice which have been tested with SOA. The SW frequency distribution is located at the low end of the

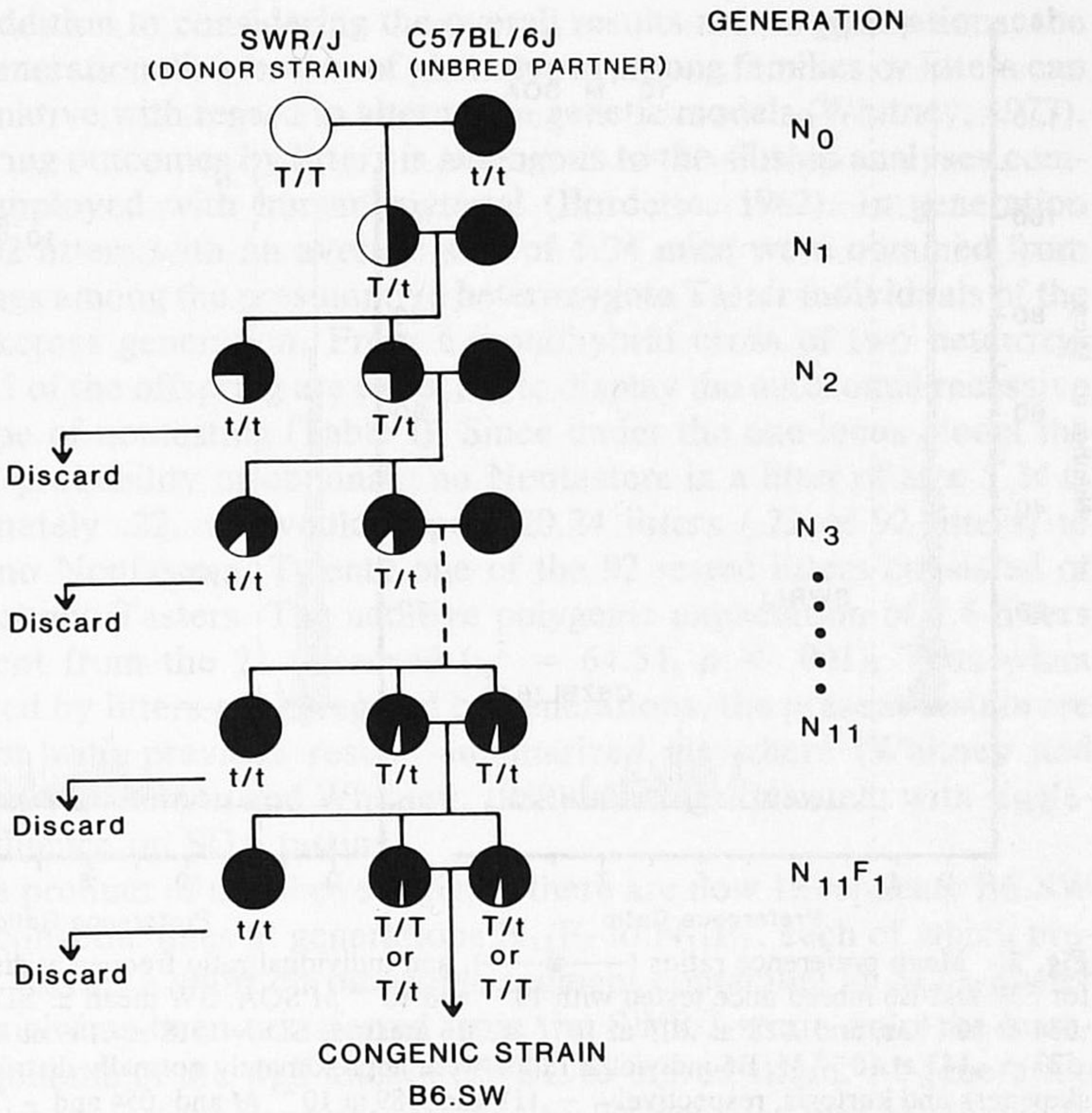

Fig. 1. Backcross (NX) system for congenic strain development involving the transfer of the autosomal dominant taster allele from the SW donor strain onto the B6 genomic background. Phenotypic Taster (T/t) mice in generations N_1–N_{10} are backcrossed to B6 inbred mice. Tasters in N_{11} (and all subsequent generations) are sib intercrossed.

preference ratio scale, reflecting essentially complete avoidance of the SOA. The B6 frequency distribution is centered near .5, corresponding to indifference between the tastant solution and the distilled water. As indicated in Fig. 2, a criterion score of about PR = 0.15 maximally discriminates SW-Taster (T) from B6-Nontaster (N) inbred mice. Although the selection criterion (PR < .10) used across segregating generations in the present study is not very different (see Fig. 2), the results below are presented relative to the PR < .15 Taster criterion since this best differentiates the progenitor inbred lines.

Table I enumerates the results obtained across lineal backcross generations as well as those from the progenitor inbreds, F_1, and the segregating F_2 and $N_{11}F_1$ generations. Table I also contains the expectations

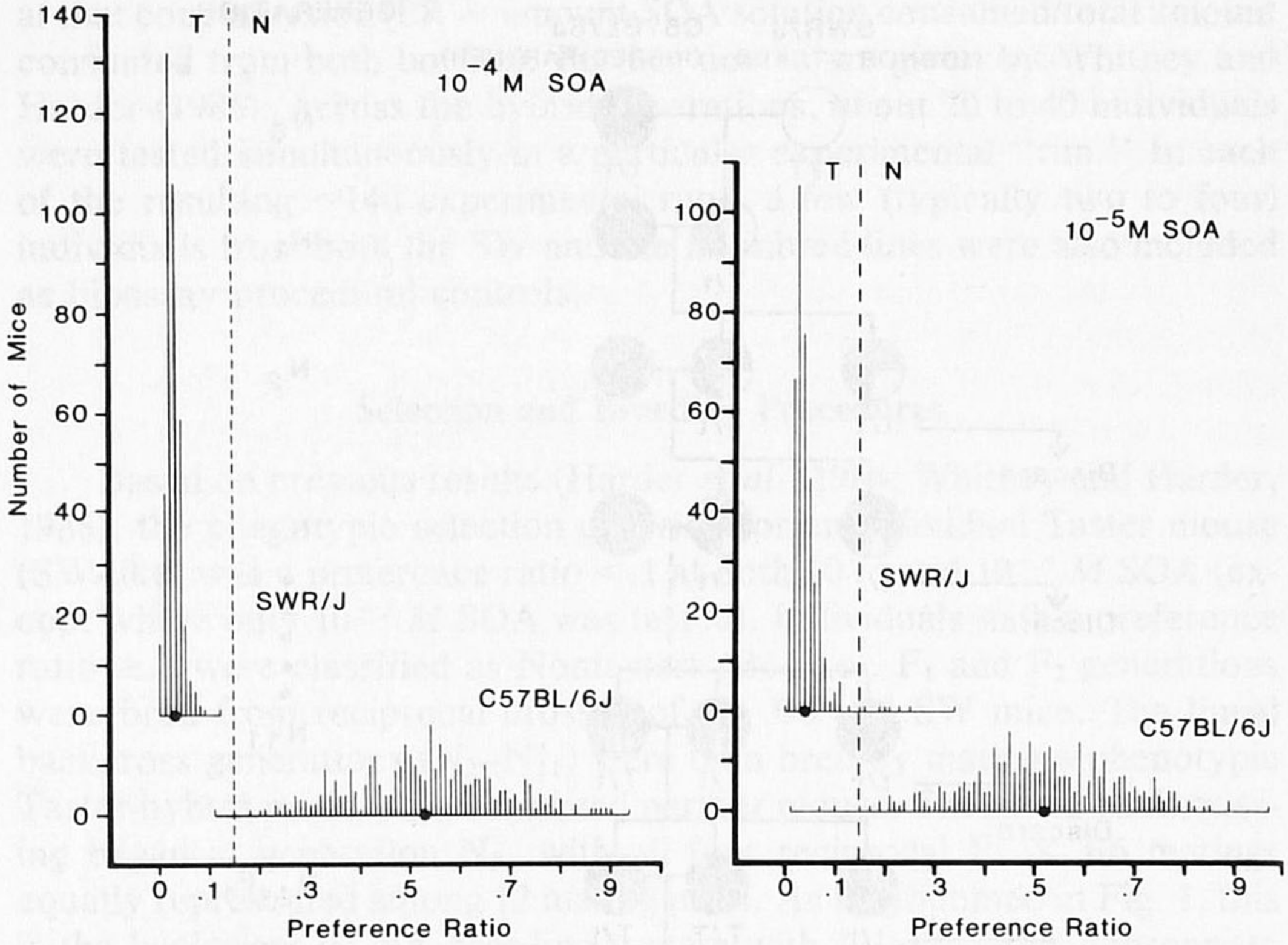

Fig. 2. Mean preference ratios (——●——), and individual ratio frequency distributions, for SW and B6 inbred mice tested with 10^{-5} and 10^{-4} *M* SOA. SW mean ± SD = .044 ± .034 at 10^{-5} *M*, and .028 ± .015 at 10^{-4} *M*. B6 mean ± SD = .518 ± .149 at 10^{-5} *M* and .527 ± .143 at 10^{-4} *M*. B6 individual ratios were approximately normally distributed, with skewness and kurtosis, respectively, −.111 and .189 at 10^{-5} *M* and .054 and −.135 at 10^{-4} *M*. The dashed vertical lines indicate the criterion preference ratio for Taster/Nontaster phenotypic classification.

for each generation under the one-locus Mendelian model and two alternative polygenic models. The alternative polygenic models, illustrated elsewhere (Whitney and Harder, 1986), involve autosomal duplicate dominance for the epistatic case and within-locus autosomal dominance for the additive case. The results from generations N_2 through N_5 at 10^{-5} *M* SOA have been previously reported (Whitney and Harder, 1986) and are included here for completeness. For each of 12 segregating generations, at both tested concentrations of SOA, the results are consistent with expectations from a one-locus Mendelian model with an autosomal allele conferring a dominant taster phenotype. Further, as listed in Table I, the results are significantly different from expectations of the alternative polygenic models except where expectations converge with those of the monogenic model.

In addition to considering the overall results across generations, the within-generation distribution of phenotypes among families or litters can be informative with regard to alternative genetic models (Whitney, 1973). Considering outcomes by litters is analogous to the sibship analyses commonly employed with human material (Burdette, 1962). In generation $N_{11}F_1$, 92 litters with an average size of 5.24 mice were obtained from sib matings among the presumptive heterozygote Taster individuals of the N_{11} backcross generation. From a monohybrid cross of two heterozygotes, .25 of the offspring are expected to display the autosomal recessive phenotype of nontasting (Table I). Since under the one-locus model the binomial probability of obtaining no Nontasters in a litter of size 5.24 is approximately .22, we would expect 20.24 litters (.22 of 92 litters) to contain no Nontasters. Twenty-one of the 92 tested litters consisted of all phenotypic Tasters. The additive polygenic expectation of 4.6 litters is different from the 21 observed ($\chi^2 = 64.54$, $p < .001$). Thus when considered by litters or aggregated by generations, the present results are congruent with previous results summarized elsewhere (Whitney and Harder, 1986; Gannon and Whitney, 1989) in being consistent with single-locus influence on SOA tasting.

As a product of the above regimen there are now 12 replicate B6.SW bilineal congenic lines at generations $N_{11}F_5$ to $N_{11}F_7$, each of which presumably carries a short chromosome segment containing an SOA-Taster allele which has been transferred from the SWR/J strain onto the background genome of the well-known C57BL/6J inbred strain. At generation N_{11}, the chromosome segment retained by Taster mice was calculated to average 18.2 cM in length. Unlinked SW genetic material totaling, on average, 1.5 cM was also expected to have been retained. This 19.7 cM of donor strain material represents about 1.2% of the ~1600-cM mouse haploid genome length. In terms of loci, residual heterozygosity would be expected in N_{11} at ~.1% of the unlinked loci for which the B6 and SW strains carry different alleles.

The most obvious value of these congenic Taster lines lies in their potential to facilitate a functional understanding of taste. At the present time taste is among the least understood of the mammalian senses. Like other domains at the interface of functional physiology, molecular biology, and behavioral phenomenology, taste is undoubtedly a rather complex sensory domain. Indeed, even with regard to the single tastant sucrose octaacetate used in the present investigation, the single-locus, two-allele model does not account for all of the phenotypic variation. For example, when testing is conducted at the near-saturation concentration of 10^{-3} *M* SOA, many inbred strains are "Tasters," even though the same strains are "Nontasters" at 10^{-4} or 10^{-5} *M* SOA (Harder *et al.*,

1984). Further, the genetics of avoidance of 10^{-3} *M* SOA can be investigated separately from the present major allelic influence at 10^{-4} and 10^{-5} *M* SOA (Gannon and Whitney, 1989), and the diagnostic avoidance phenotypes are themselves modifiable by manipulation of tastant exposures during ontogeny (Harder *et al.*, 1989). Nevertheless, or perhaps more appropriately, because of, the existing complexities of taste, this model system consisting of the presence or absence of a single-major allele influence, expressed on an otherwise near-homogeneous genetic background, may represent a simplification which will facilitate functional analysis and integration across levels from the molecular to the behavioral. Congenic taster mice could contribute as much to the future understanding of the chemical senses as congenic resistant mice have already contributed to the understanding of immunology.

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Sucrose Octaacetate Tasting in a Heterogeneous Population of CFW Mice

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Three experiments investigated the genetic underpinnings of the sucrose octaacetate (SOA) avoidance-indifference dimorphism that exists among outbred CFW mice. In the first experiment, results from 687 subjects across three generations of segregation were consistent with predictions from a single-autosomal, two-allele model, with dominance for the avoidance (Taster) phenotype. In the second experiment, heterogeneous CFW Tasters and Nontasters were mated with SWR/J (Taster) and C57BL/6J (Nontaster) inbred mice. The SWR and CFW mice are both derived from Swiss mice, and the results were consistent with the possibility that the Taster animals share an allele which is identical by descent. The second and third experiments also investigated sensitivity to SOA across an extended range of concentrations. Nontaster CFWs avoided SOA at the near-saturation 10^{-3} M concentration but did not avoid any weaker concentrations. Taster CFWs avoided all concentrations down to approximately 10^{-6} M SOA.

KEY WORDS: taste genetics; chemosensory genetics; sucrose octaacetate; mouse; *Mus domesticus*; single locus.

INTRODUCTION

Research involving gustatory sensitivity to various bitter substances (e.g., phenylthiourea, quinine, strychnine, and sucrose octaacetate) has implicated monogenic modes of inheritance operating in certain populations of mice (Klein and DeFries, 1970; Lush, 1981a, 1982, 1984; Whitney and

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Harder, 1986). A substantial amount of research effort has been devoted to the synthetic compound, sucrose octaacetate (SOA).

Individual differences in SOA sensitivity among mice were initially reported by Warren (1963). In 1970, Warren and Lewis tested several inbred strains and found that in two-bottle preference tests, CFW/NIH mice avoided water containing SOA. Subsequent backcross results were consistent with the hypothesis that SOA detection involves a single autosomal locus with a dominant "taster" allele.

After an interim of approximately 10 years, Lush (1981a) screened 31 inbred strains of mice (including a CFW strain) for SOA sensitivity. Individual testing of his CFW mice revealed no SOA avoiders. Only the SWR strain manifested an aversion to SOA. Crosses involving SWRs revealed Taster vs. Nontaster ratios consistent with the expectation from a single-autosomal gene model. Lush (1981b) named the two presumptive alleles Soa^a (aversion, dominant) and Soa^b (blind, recessive). Subsequent investigations confirmed and extended Lush's findings concerning the SWR strain (Harder *et al.*, 1984; Shingai and Beidler, 1985; Whitney and Harder, 1986).

The apparent discrepancy involving CFW mice, however, lingered unexplained in the literature until recently. Initially, CFWs were reported by Warren and Lewis (1970) to be avoiders or "Tasters" of SOA, but later, they were categorized as SOA nonavoiders or "Nontasters" (Lush, 1981a). In 1986, Whitney and Harder discovered SOA Tasters and Nontasters in an outbred CFW line, Crl:CFW(SW)BR. A genetic component to SOA sensitivity differences among outbred CFW mice was implicated in that Taster and Nontaster phenotypes tended to breed true. They proposed that the previous disparate findings regarding SOA sensitivity of CFW mice could be attributed to the use of genetically distinct CFW lines. CFW mice were derived from a heterogeneous Swiss stock, and hence, sublines may not be genetically identical (Lynch, 1969; Sher, 1974; Staats, 1981).

The present experiments were undertaken to characterize SOA sensitivity among CFW animals. The first experiment explored the genetic transmission of SOA sensitivity differences that exist among outbred CFWs. The second experiment investigated the allelic mechanisms operating in heterogeneous CFW mice (Tasters and Nontasters) relative to those of inbred SWR/J (Taster) and C57BL/6J (Nontaster) mice. Sensitivity ranges for CFW Taster and Nontaster phenotypes were ascertained in the third experiment.

EXPERIMENT 1

The present experiment was conducted to elucidate the genetic influence on SOA sensitivity among outbred CFW animals. The single-

autosomal locus, dominant-Taster model was explicitly tested to determine whether the genetic underpinnings of SOA sensitivity among CFW animals parallel those in SWR/J mice.

Method

Subjects. A total of 687 outbred descendants of a progenitor stock, Crl:CFW(SW)BR (hereinafter CFW), purchased from Charles River Laboratories (Portage, Mich.) was tested. All animals were maintained in a manner described elsewhere (Whitney and Harder, 1986). Individuals were tested at 40–50 days of age in stainless-steel cages (10 × 24 × 13 cm) with metal clips allowing the placement of inverted, graduated 25-ml glass cylinders on the front of the cages.

Solutions. SOA (Sigma Chemical Company) solutions were prepared with distilled water and, due to the relative insolubility of SOA, were heated below the boiling point. Both SOA and control (distilled water) solutions were presented to the animals at room temperature.

Testing Procedures. Subjects were administered two-bottle preference tests (SOA vs. distilled water) in which 10^{-5} *M* SOA preceded 10^{-4} *M* SOA, each concentration spanning 48 h. Testing was conducted as previously described by Whitney and Harder (1986). No preference for either solution would result in a preference ratio of .50. Complete avoidance of the SOA solution would result in a ratio of .00. The criterion used for Taster categorization was a preference ratio below .10. Animals with preference ratios of .10 and above were classified as Nontasters. This criterion was chosen on the basis of maximally distinguishing between SWR/J (Taster) and C57BL/6J (Nontaster) mice tested in our laboratory as well as distinguishing between the two CFW phenotypic distributions found by Whitney and Harder (1986).

Breeding Procedures. Progenitors of the first generation of animals were CFW mice maintained in our laboratory, previously tested for SOA avoidance, and classified as either Tasters or Nontasters. Crosses of phenotypic Taster (T) and Nontaster (N) CFW animals yielded a first generation (G_1). All offspring were subsequently tested and categorized, with selected Tasters and Nontasters parenting the next generation. Second-generation (G_2) animals were derived from the following pairings: T × T, T × N, N × T, and N × N. A test cross was employed to determine the genotype of one G_1 animal which did not avoid the SOA in the initial testing session but did avoid the solution in a post hoc, second preference test. The original classification of the animal as a Nontaster was suspect due to consistent SOA avoidance by all siblings.

A third generation (G_3) of animals was bred (identical to G_2 matings) utilizing G_2 mice as progenitors. Offspring from the previously mentioned test cross were also used to parent the third generation.

Table I. Percentages of Phenotypic Sucrose Octaacetate (SOA) Tasters Among CFW G_1 Animals from Eleven Taster × Nontaster Crosses

Mating pair No.	No. of offspring	% Tasters exp.	% Tasters observed	
			10^{-5} *M* SOA	10^{-4} *M* SOA
1	17	100	100	100
2	15	100	100	100
3	11	100	100	100
4	22	100	91 ($p = .24$)[a]	100
5	22	100	91 ($p = .24$)[a]	100
6	20	100	85 ($p = .12$)[a]	100
7	17	100	94 ($p = .50$)[a]	100
8	18	100	94 ($p = .50$)[a]	94($p = .50$)[a]
9	24	50	67 ($p > .10$)[b]	67($p > .10$)[b]
10	16	50	38 ($p > .30$)[b]	31($p > .10$)[b]
11	20	50	40 ($p > .30$)[b]	40($p > .30$)[b]

[a] Fisher exact probability test.
[b] Chi-square goodness-of-fit test.

Results and Discussion

Phenotypic proportions for 202 G_1 animals are summarized in Table I. Eleven pairings between SOA Tasters and Nontasters (each producing two litters) were expected to yield one of two predicted Taster:Nontaster ratios in the resulting offspring. According to a single-locus model with an autosomal dominant allele promoting SOA avoidance, parental Tasters should be either homozygous dominant or heterozygous. Therefore, ratios of 100:0 or 50:50 should result from pairings of Tasters and Nontasters (presumptive homozygous recessive) if the model is correct.

Each expected percentage in Table I reflects a post hoc "best fit" of one of the two hypothesized outcomes to the data. Across both concentrations, the observed numbers of Taster offspring are statistically consistent with one or the other prediction derived from a single-locus, two-allele model. A two-factor analysis of variance (ANOVA) revealed no significant main effects or interactions for sex or concentration. The apparent differences in phenotypic percentages across the two concentrations in Table I result from some 10^{-4} *M* Tasters just exceeding the .10 Taster criterion when tested with the weaker 10^{-5} *M* SOA.

For those mated pairs (T × N) producing 100% Tasters, it was presumed that the tasting parent was homozygous dominant and therefore all progeny from those pairs were presumed to be heterozygous. Crosses producing a percentage of Tasters not significantly different from 50% must have had a heterozygous Taster parent if the initial model is correct.

Therefore, the offspring resulting from such crosses were assigned a presumptive genotype of either homozygous recessive (tt) if a Nontaster or heterozygous (Tt) if classified as a Taster after two-bottle preference testing.

A post hoc test cross was carried out on a G_1 animal from mating pair 8 (Table I). This individual initially was classifed as a Nontaster at both concentrations. However, all siblings ($n = 17$) avoided the SOA at both concentrations. Upon retesting with both concentrations, the animal in question avoided the tastant, while three other previously tested Nontasters from the same generation did not. Test crossing this animal to a Nontaster produced progeny ($n = 28$) in which 43% avoided 10^{-5} *M* SOA and 50% avoided 10^{-4} *M* SOA. These findings were consistent with the 50% Tasters expected from a (Tt) × (tt) cross [$\chi^2(1) = .57$, $p > .40$]. Apparently this animal possessed a Taster genotype but failed to exhibit the avoidance phenotype on initial tastant encounter. A second generation (G_2) was bred to test further the genetic model underlying SOA detection among CFWs. Ten mated pairs (2–4 litters/pair) were established using G_1 mice as progenitors: (Tt) × (Tt), (Tt) × (tt), (tt) × (Tt), and (tt) × (tt). A priori expected percentages of Nontasters for these crosses were 25, 50, 50, and 100%, respectively.

Data from the reciprocal T × N and N × T crosses were combined because no significant differences in phenotypic ratios were revealed at 10^{-5} *M* SOA [$\chi^2(1) = 1.47$, $p > .20$] or 10^{-4} *M* SOA [$\chi^2(1) = .197$, $p > .60$]. Phenotypic results of 237 G_2 offspring from the 10 pairs were consistent with expected values derived from a single-locus, two-allele model for each type of cross (see Figs. 1A–C, insets).

Figures 1A–C show the preference ratio frequency distributions for each G_2 pairing type at 10^{-4} *M* SOA. Two-factor ANOVAs yielded no significant main effects or interactions for concentration or sex across all three types of crosses. The T × T ($n = 84$) and T × N ($n = 59$) progeny each displayed a bimodal distribution of preference ratios. The T × T and T × N distributions each contain a group of mice with preference ratios below .10 (reflecting avoidance of SOA) as well as a group of mice with preference ratios distributed around .50 (reflecting indifference). The N × N ($n = 94$) preference ratio frequency distribution approximates a normal distribution (mean = .50) and resembles the second portion of the bimodal distributions produced by T × T and T × N pairings.

A third generation (G_3), obtained from G_2 Nontasters and test cross-derived Tasters, served two purposes. One was to replicate results from the previous generation, and the other was to follow the test cross-derived animals for another segregating generation. Nine pairings (one T × T, three T × N, two N × T, and three N × N pairs) produced 220 progeny.

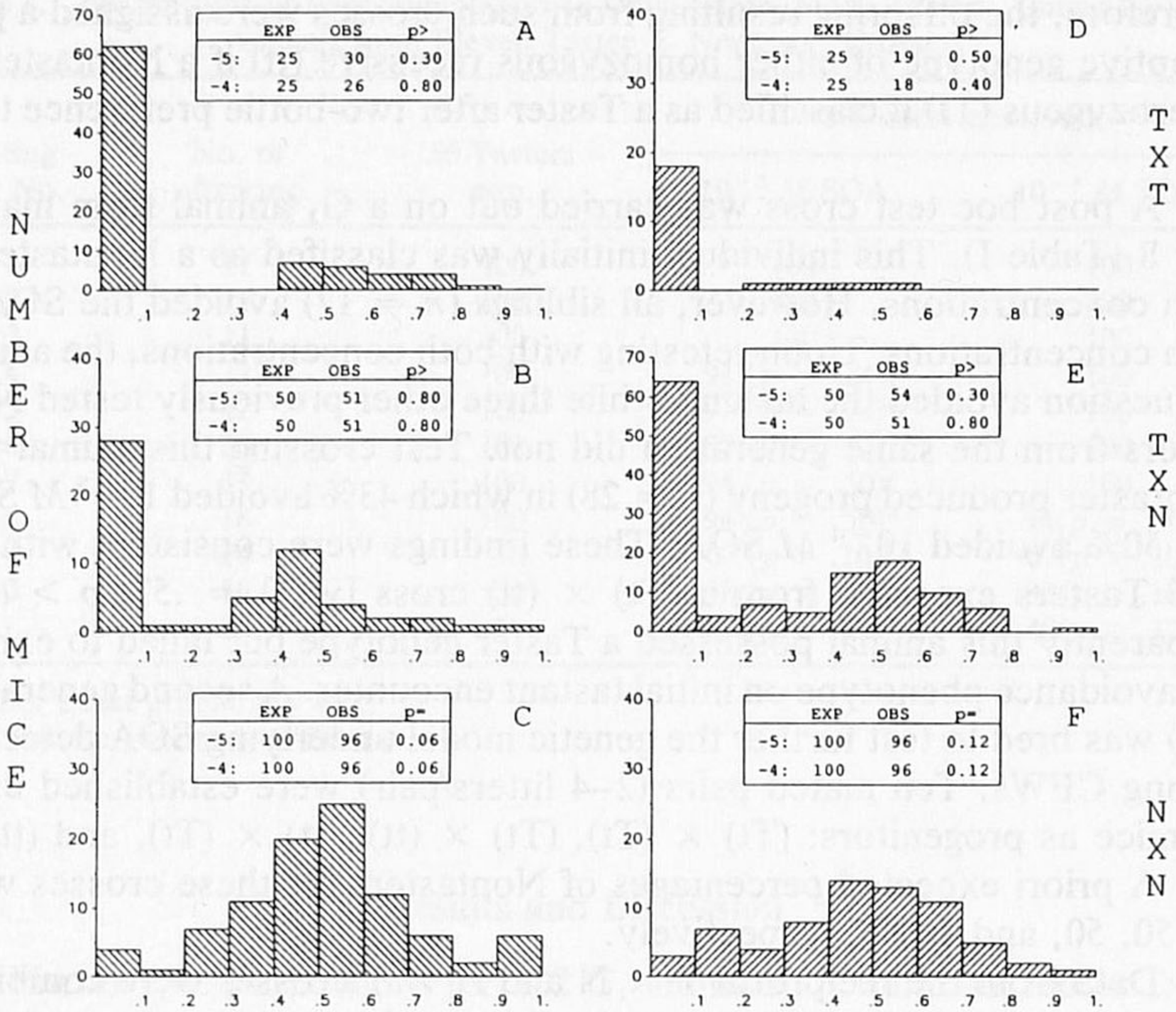

Fig. 1. Preference ratio frequency distributions for CFW mice tested with 10^{-4} *M* SOA. Results of second-generation (G_2) animals from T × T (genotype Tt) ($n = 84$), T × N (genotype tt) ($n = 59$), and N × N ($n = 94$) pairings are shown in A–C and those of third-generation (G_3) animals are presented in D–F ($n = 22$, 130, and 68, respectively). Insets show observed percentages of Nontasters and those expected for 10^{-4} and 10^{-5} *M* SOA according to a single-locus model. Analyses were performed using chi-square goodness-of-fit tests or Fisher exact probability tests.

All cross types produced offspring phenotypic ratios consistent with those expected of the single-locus model (Figs. 1D–F, insets). Once again, reciprocal crosses were combined (labeled T × N) due to the absence of significant reciprocal effects at 10^{-5} *M* SOA [$\chi^2(1) = .82$, $p > .30$] or at 10^{-4} *M* SOA [$\chi^2(1) = .14$, $p > .70$]. The G_3 preference ratio distributions (Figs. 1D–F) are similar to the distributions produced by G_2 animals, with T × T and T × N crosses yielding bimodal distributions and N × N progeny approximating a normal distribution (mean = .47).

Three generations of Mendelian breeding produced SOA avoidance/nonavoidance phenotypic ratios among heterogeneous CFWs consistent with a single-autosomal locus, two-allele mode of inheritance (the taster allele dominant to the nontaster allele). This finding parallels the model

accounting for SOA avoidance among progeny of SWR/J mice crossed to a variety of other inbred lines (Lush, 1981a; Whitney and Harder, 1986).

Frequency distributions of individual preference ratios were either bimodal (T × T and T × N) or unimodal (N × N) across all generations (Figs. 1A–F). Taster distributions were characterized by a mean of .03 and a variance of .0003, whereas Nontaster distributions had a mean approximating .50 and a larger variance of approximately .04. A small proportion of apparent Tasters would be expected from N × N crosses, namely, that proportion at the extreme lower end of an approximately normal distribution. The combined G_2 and G_3 Nontaster preference ratio distribution was symmetrical at both extremes, with 4% of the animals having ratios above .90 as well as below .10 for 10^{-4} *M* SOA (mean = .49, SD = .20). Assuming a normal distribution, the expected proportion of subjects falling below 0.10 or above 0.90 is 2.6%, not statistically different from the 4% obtained [$\chi^2(1) = 1.90$, $p > .10$].

Of 687 animals, one "misclassification" was discovered. It was not determined whether procedural error or environmental factors were responsible, although genetic factors did not seem to be involved. The present experiment does not directly address the overall reliability of the SOA-avoidance phenotype among CFW mice, although unreliable phenotypes would not likely produce such consistent phenotypic ratios across multiple generations. Short-term (6-day) testing in our laboratory of SWR/J and SWR/J × C57BL/6J hybrids at 10^{-5} and 10^{-4} *M* SOA resulted in reliable SOA avoidance across days.

There was an indication of incomplete dominance or reduced expressivity when testing was conducted with the less concentrated 10^{-5} *M* SOA. Some 10^{-4} *M* SOA Tasters marginally exceeded the Taster criterion at 10^{-5} *M* SOA, suggesting a slightly less stringent avoidance of weaker SOA concentrations. This deviation from the expected proportion of CFW Tasters was not significant (chi-square analyses), and no significant concentration effect was indicated by ANOVA. Experiment 2, below, was designed to investigate further phenotypic and genotypic aspects of SOA tasting among animals of diverse origin.

EXPERIMENT 2

The present outbred CFW stock and the SWR/J inbred strain were both descended from nine albino mice received by Lynch from Switzerland in 1926 (Whitney and Harder, 1986). Thus, it was reasonable to hypothesize that the SOA-taster allele, apparently segregating in the CFW stock, was identical by descent to the taster allele homozygous in the SWR/J strain. Alternatively, it was possible that the shared SOA-Taster

phenotype was fortuitous and caused by separate alleles, perhaps at different loci in the two lines. To investigate the relationship of alleles among these lines, CFW Tasters were crossed with both Taster and Nontaster inbred mice. Any discrepancy from predicted phenotypic outcomes using an identical-taster allele, single-locus model would suggest that CFW-Taster and SWR mice do not share *Soa*-locus alleles identical by descent.

The second purpose of the experiment was to gain information regarding the expression of the SOA-Taster and Nontaster phenotypes resulting from crosses of CFW genomes to those of known inbred lines. Specifically, three questions were of interest: (1) Given that heterozygotes from SWR/J crosses display one of two alternative degrees of dominance when crossed to different inbred lines (Harder and Whitney, 1985; Whitney and Harder, 1986), do SWR × CFW heterozygotes display one of the already familiar dominance patterns? (2) Given that taster alleles from SWR inbreds have an already characterized phenotypic expression when crossed with C57BL/6J (Nontaster) inbreds (Whitney and Harder, 1986), will taster alleles from the CFW stock display a similar phenotypic pattern in crosses to the same Nontaster inbred? and (3) Given that various SOA Nontaster inbred lines respond differently to the near-saturated 10^{-3} *M* SOA (Harder *et al.*, 1984), how do CFW Nontasters and their Nontaster hybrid progeny (CFW Nontaster × C57BL/6J) respond to 10^{-3} *M* SOA?

Method

Subjects. Animals ($n = 196$) were obtained from pairings of CFW mice (Tasters and Nontasters) to SWR/J (Taster) and C57BL/6J (Nontaster) mice. Subjects also included 18 CFW(N) mice. Parental inbred mice were bred in our laboratory from animals purchased from Jackson Laboratories (Bar Harbor, Maine). CFW mice were selected from the first experiment (G_1 and G_2), above. All animals were reared as described previously.

Procedures. Thirteen mated pairs produced one to four litters each. CFW Tasters (derived from T × N and N × T crosses and presumed heterozygous) were mated to SWR/J mice (homozygous dominant; four pairs) and C57BL/6J (homozygous recessive; three pairs). CFW Nontasters were also mated to SWR/J (two pairs) and C57BL/6J mice (four pairs).

All offspring were tested as specified in the previous experiment. To investigate directional dominance between the Nontasting genomes, 30 C57BL/6J × CFW(N) progeny were tested with 10^{-3} *M* SOA, as were 18 CFW(N) mice.

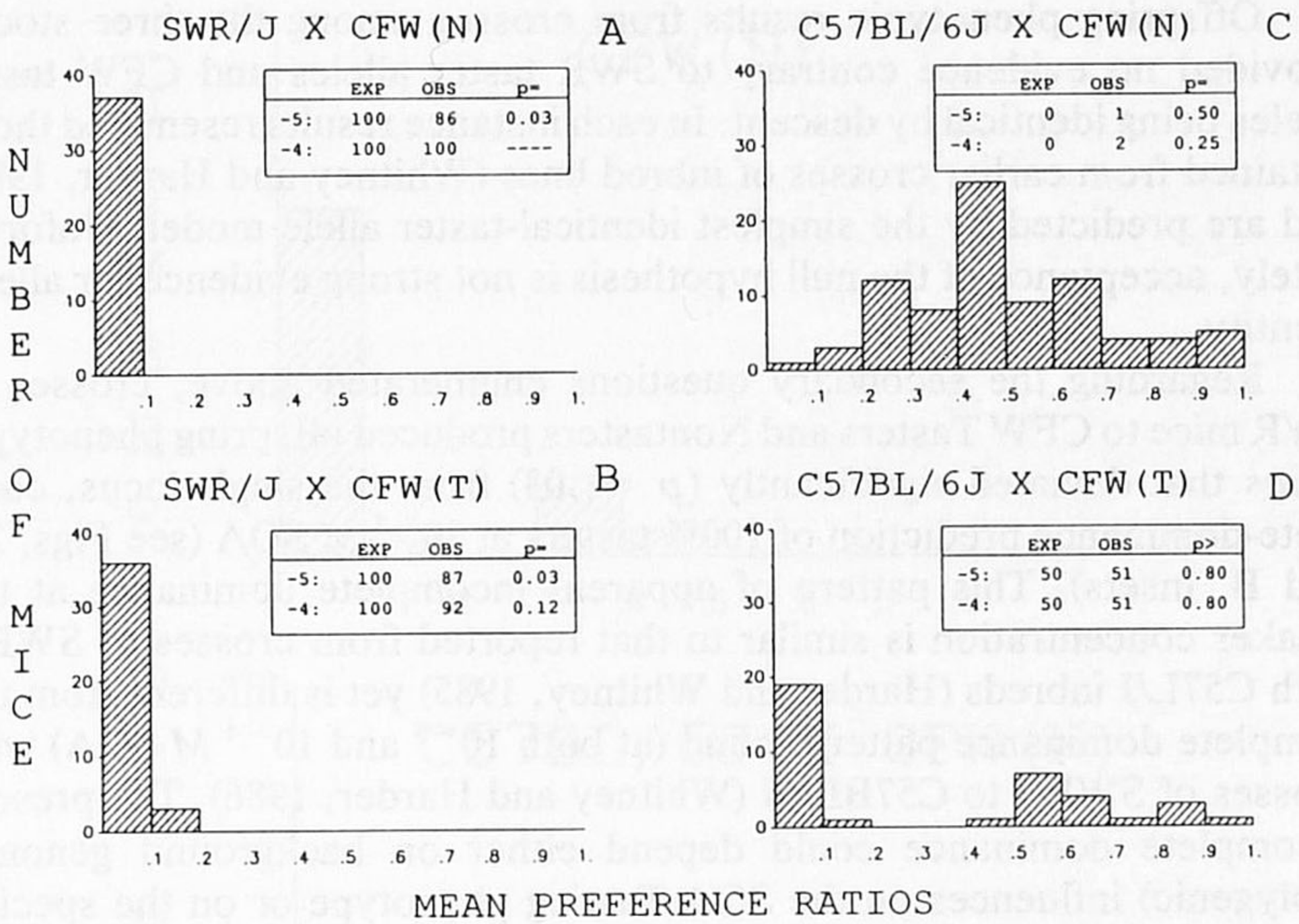

Fig. 2. Preference ratio frequency distributions for (A) SWR/J (genotype TT) × CFW(N) (genotype tt) (n = 37), (B) SWR/J × CFW(T) (genotype Tt) (n = 39), (C) C57BL/6J (genotype tt) × CFW(N) (n = 83), and (D) C57BL/6J × CFW(T) (n = 37) progeny tested with 10^{-4} M SOA. Observed and expected percentages of Tasters, along with significance levels (chi-square or Fisher exact probability tests) for 10^{-5} and 10^{-4} M SOA are shown in the insets.

Results and Discussion

Expected proportions of Tasters and Nontasters were derived a priori for a single-locus, two-allele model with complete taster-allele dominance. When tested with 10^{-4} M SOA, all observed phenotypic ratios were consistent with expected ratios (see Fig. 2, insets).

Preference ratio frequency distributions for SWR/J × CFW and C57BL/6J × CFW progeny for 10^{-4} M are shown in Fig. 2. With little variance, hybrids resulting from both SWR/J × CFW(N) and SWR/J × CFW(T) crosses gave the expected Taster distribution (Figs. 2A and B). Virtually all animals had preference ratios below .10. Crosses of C57BL/6J × CFW(N) produced progeny with a typical Nontaster distribution (Fig. 2C). Heterozygous CFW Tasters, when mated to C57BL/6J mice, produced progeny whose preference ratios for 10^{-4} M SOA were bimodally distributed (Fig. 2D). This distribution resembles the distribution of CFW T × N progeny in G_2 and G_3 of Experiment 1 (Figs. 1B and E), as well as those of segregating generations from crosses involving SWR/J and C57BL/6J inbreds (Whitney and Harder, 1986).

Offspring phenotypic results from crosses among the three stocks provided no evidence contrary to SWR taster alleles and CFW taster alleles being identical by descent. In each instance results resembled those obtained from earlier crosses of inbred lines (Whitney and Harder, 1986) and are predicted by the simplest identical-taster allele model. Unfortunately, acceptance of the null hypothesis is not strong evidence for allelic identity.

Regarding the secondary questions enumerated above, crosses of SWR mice to CFW Tasters and Nontasters produced offspring phenotypic ratios that deviated significantly ($p = .03$) from the single-locus, complete-dominance prediction of 100% tasters at 10^{-5} *M* SOA (see Figs. 2A and B, insets). This pattern of apparent incomplete dominance at the weaker concentration is similar to that reported from crosses of SWR/J with C57L/J inbreds (Harder and Whitney, 1985) yet is different from the complete dominance pattern found (at both 10^{-5} and 10^{-4} *M* SOA) with crosses of SWR/J to C57BL/6J (Whitney and Harder, 1986). The present incomplete dominance could depend either on background genomic (polygenic) influences on the SOA-Tasting phenotype or on the specific nontasting allele provided by the CFW parent.

The tasting allele provided by the CFW stock apparently confers complete dominance (for both 10^{-5} and 10^{-4} *M* SOA tasting) on heterozygotes from C57BL/6J × CFW(T) crosses (see Fig. 2D, inset). This pattern is identical to results obtained for the taster allele derived from SWR/J inbred animals (Whitney and Harder, 1986).

Figure 3 presents the results from testing Nontasters with the near-saturated 10^{-3} *M* SOA concentration. CFW(N) mice displayed a mean preference ratio of .12, which significantly differed from .50 or indifference [$t(17) = -12.21$, one-tailed $p < .0005$]. CFW(N) animals also significantly differed from C57BL/6J × CFW(N) mice at the 10^{-3} *M* concentration [$t(46) = -5.37$, one-tailed $p < .0005$].

In previous tests, 8 of 10 nontasting inbred strains were sensitive to 10^{-3} *M* SOA (Harder *et al.*, 1984). The two lines which did not reliably avoid this concentration were C57L/J and C57BL/6J. In the present experiment, C57BL/6J × CFW(N) hybrids displayed directional dominance of the insensitive, C57-like phenotype (Fig. 3). Thus, 10^{-3} *M* SOA avoidance among animals not carrying the taster allele could be due to polygenic influences or could result from alternative alleles additional to the 10^{-5} and 10^{-4} *M* SOA taster allele identified above and previously (Whitney and Harder, 1986). Additional investigation would be required to characterize the genetic influence on sensitivity to high concentrations of SOA in the absence of the 10^{-4} *M* SOA taster gene.

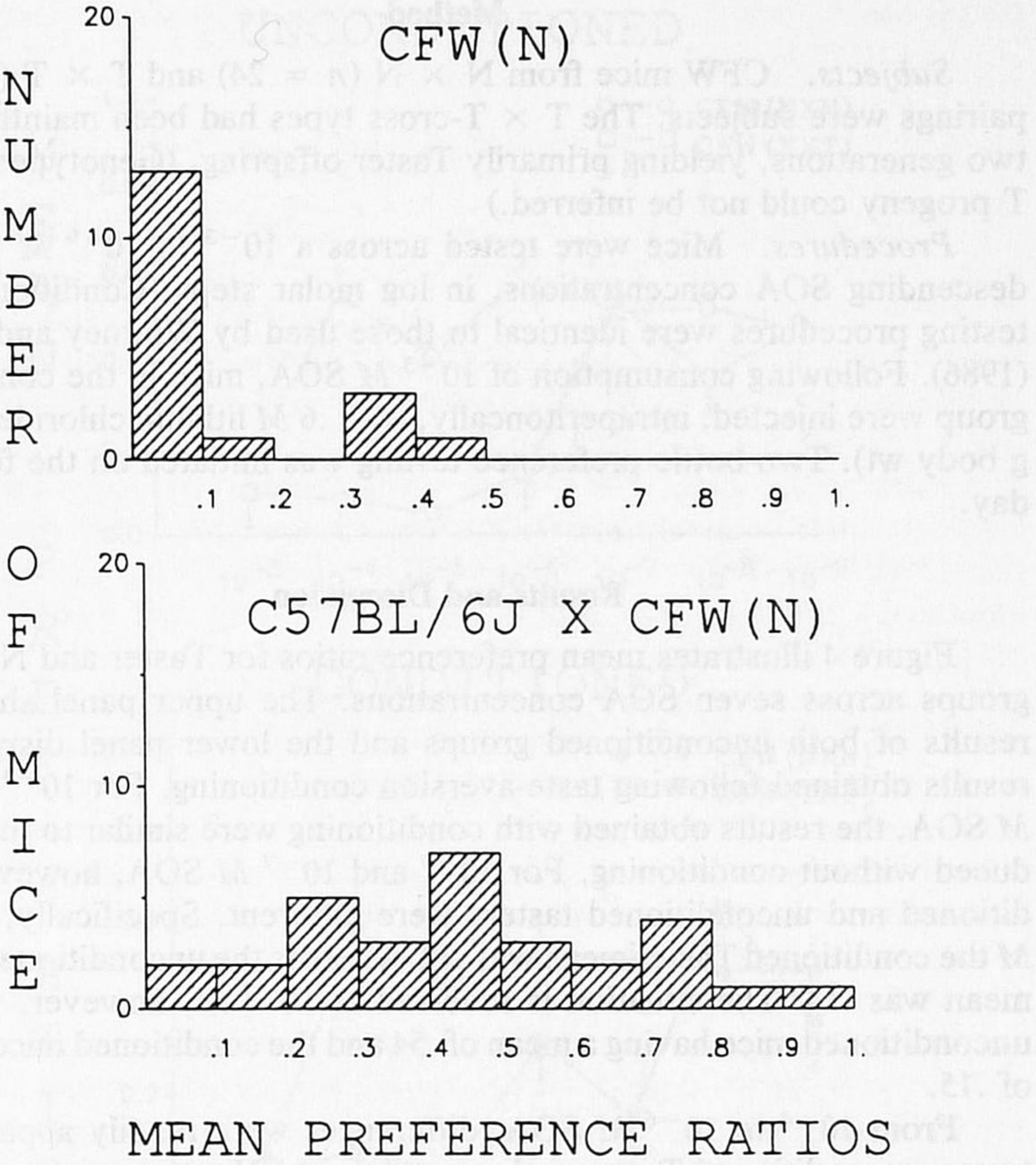

Fig. 3. Comparison of preference ratio distributions for CFW(N) ($n = 18$) and C57BL/6J × CFW(N) ($n = 30$) animals tested with 10^{-3} *M* SOA. Group mean preference ratios were .12 and .45, respectively.

EXPERIMENT 3

It is obvious from the above results and previous research (Harder *et al.*, 1984) that concentration plays a crucial role in the response of mice to SOA. The ensuing experiment investigated the role of concentration with regard to CFW Taster and Nontaster phenotypes. A conditioned taste-aversion paradigm (entailing pairing the tastant with the effects of a noxious stimulus) was used to enhance the animals' display of their detection of lower SOA concentrations (Barker *et al.*, 1977).

Method

Subjects. CFW mice from N × N ($n = 24$) and T × T ($n = 22$) pairings were subjects. The T × T-cross types had been maintained for two generations, yielding primarily Taster offspring. (Genotypes of T × T progeny could not be inferred.)

Procedures. Mice were tested across a 10^{-3} to 10^{-9} *M* range of descending SOA concentrations, in log molar steps. Conditioning and testing procedures were identical to those used by Whitney and Harder (1986). Following consumption of 10^{-3} *M* SOA, mice in the conditioned group were injected, intraperitoneally, with .6 *M* lithium chloride (.02 ml/g body wt). Two-bottle preference testing was initiated on the following day.

Results and Discussion

Figure 4 illustrates mean preference ratios for Taster and Nontaster groups across seven SOA concentrations. The upper panel shows the results of both unconditioned groups and the lower panel displays the results obtained following taste-aversion conditioning. For 10^{-3} to 10^{-5} *M* SOA, the results obtained with conditioning were similar to those produced without conditioning. For 10^{-6} and 10^{-7} *M* SOA, however, conditioned and unconditioned tasters were different. Specifically, at 10^{-6} *M* the conditioned Taster mean was .32, whereas the unconditioned Taster mean was .14. The situation is reversed at 10^{-7} *M*, however, with the unconditioned mice having a mean of .54 and the conditioned mice a mean of .15.

From 10^{-4} to 10^{-6} *M* SOA, differences were readily apparent between unconditioned Taster and unconditioned Nontaster groups. Interestingly, for 10^{-3} *M*, the two phenotypes similarly avoid the tastant. This pattern of avoidance of 10^{-3} *M* SOA and indifference at weaker concentrations has been reported for variouis Nontaster mouse strains (Harder *et al.*, 1984).

Data were analyzed parametrically [$F_{max}(10) = 2.95$, $p > .05$] using a Genotype × Treatment × Concentration ANOVA. Results indicated a significant main effect of Genotype [$F(1/42) = 53.51$, $p < .0001$] and Concentration [$F(6/252) = 17.97$, $p < .0001$], as well as a significant G × C interaction [$F(6/252) = 8.04$, $p < .0001$]. These results suggest that the CFW Taster and Nontaster phenotypes differ across a limited number of SOA concentrations, namely, 10^{-4} and 10^{-5} *M* SOA. Both groups of animals avoided the stronger, 10^{-3} *M*, concentration and were indifferent to the weaker 10^{-7} through 10^{-9} *M* concentrations. Conditioning procedures, utilized in an attempt to uncouple indifference due to insensi-

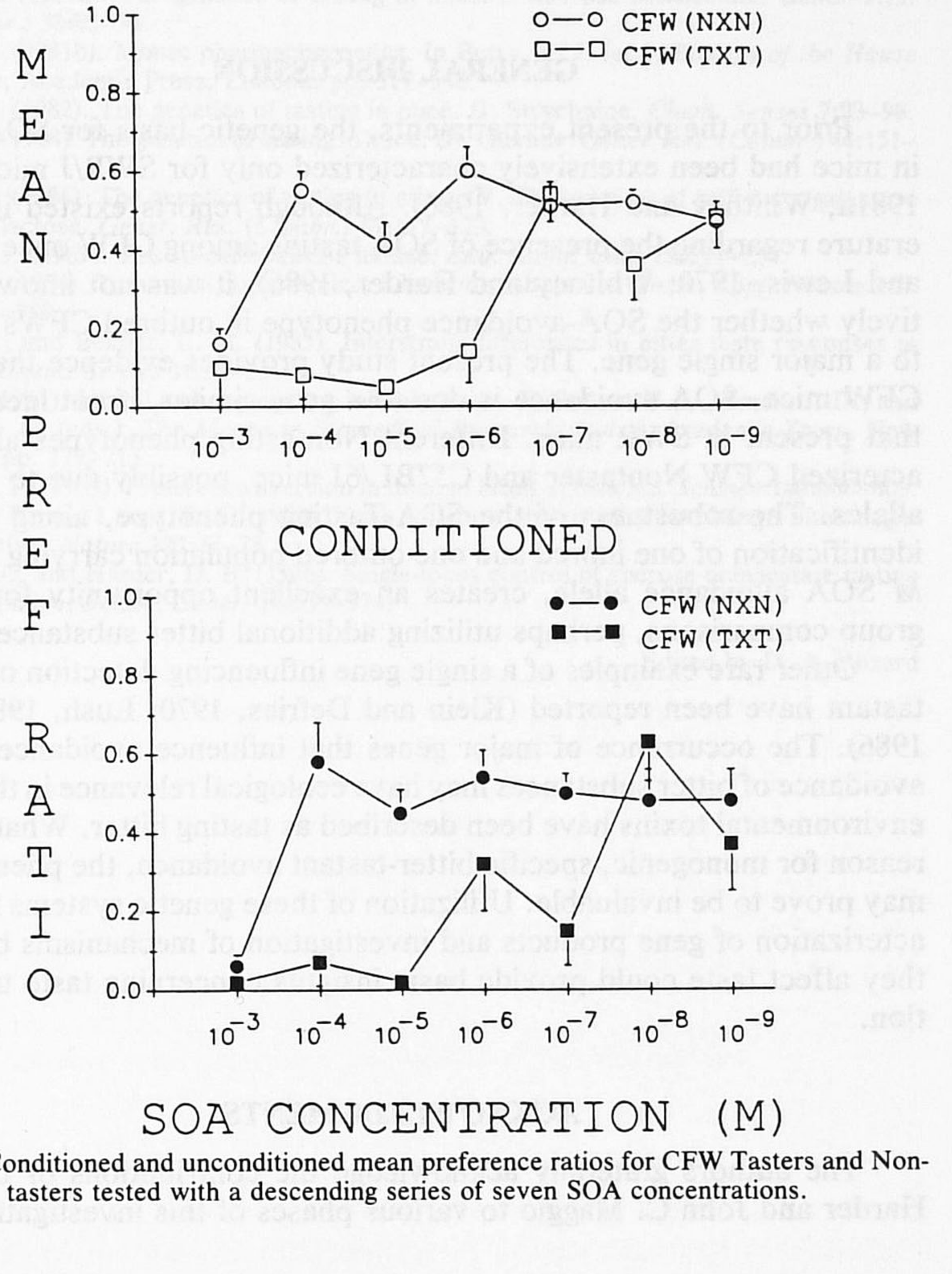

Fig. 4. Conditioned and unconditioned mean preference ratios for CFW Tasters and Non-tasters tested with a descending series of seven SOA concentrations.

tivity from that due to insufficient motivation, resulted in preference ratios similar to those of unconditioned mice.

The fact that the unconditioned Taster mean was lower than the conditioned Taster mean at 10^{-6} *M* SOA suggests that this concentration may be at or near detection threshold for these animals, thereby resulting in increased variance. As shown in Fig. 4, fluctuations in preference ratio

means for the unconditioned and conditioned Tasters were apparent from 10^{-6} to 10^{-9} *M* SOA.

GENERAL DISCUSSION

Prior to the present experiments, the genetic basis for SOA tasting in mice had been extensively characterized only for SWR/J mice (Lush, 1981a; Whitney and Harder, 1986). Although reports existed in the literature regarding the presence of SOA tasting among CFW mice (Warren and Lewis, 1970; Whitney and Harder, 1986), it was not known definitively whether the SOA-avoidance phenotype in outbred CFWs was due to a major single gene. The present study provides evidence that among CFW mice, SOA avoidance is due to a gene similar, if not identical, to that present in SWR mice. Different Nontasting phenotypes also characterized CFW Nontaster and C57BL/6J mice, possibly due to different alleles. The robustness of the SOA-Tasting phenotype, along with the identification of one inbred and one outbred population carrying the 10^{-4} *M* SOA avoidance allele, creates an excellent opportunity for further group comparisons, perhaps utilizing additional bitter substances.

Other rare examples of a single gene influencing detection of a bitter tastant have been reported (Klein and Defries, 1970; Lush, 1982, 1984, 1986). The occurrence of major genes that influence avoidance or nonavoidance of bitter substances may have ecological relevance in that many environmental toxins have been described as tasting bitter. Whatever the reason for monogenic, specific bitter-tastant avoidance, the phenomenon may prove to be invaluable. Utilization of these genetic systems for characterization of gene products and investigation of mechanisms by which they affect taste could provide basic insights concerning taste transduction.

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Behavioral and Reproductive Differences in Mice as a Function of Inbreeding

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Twenty brother–sister pairs of mice were randomly selected from a genetically heterogeneous population of mice to begin a schedule of inbreeding that lasted for six generations (F = *0 to* F = *.732*). *We examined a number of indices of reproductive behavior and found that all declined as a function of inbreeding. Specifically, there was a consistent decline in the number of fertile matings, in the number of offspring that survived to weaning, and in the weight of the pups at the time of weaning (21 days of age). We also examined a number of behaviors with the following results: there was a systematic increase in the number of trials required to learn an active avoidance task and a consistent decrease in the number of trials required to extinguish this habit. We observed a statistically significant difference in the retention of a passive avoidance habit, but these results were quite variable and not consistent across generations of inbreeding. Finally, we observed that inbreeding had little effect on measures of locomotor behavior.*

KEY WORDS: mice; inbreeding; fitness; reproduction; learning; activity.

INTRODUCTION

The goal of the experiments reported below was to examine the effects of a systematic schedule of inbreeding on certain measures of reproduction and behavior. To determine if the particular behavioral phenotypes we had chosen to measure were closely related to fitness, we examined various measures of learning and locomotor activity for evidence of inbreeding depression.

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Fisher's "fundamental theorem of natural selection" (1958) states that the change in fitness in the population mean is determined by the amount of additive genetic variance in fitness. The proportion of total phenotypic variance determined by the additive genetic variance is heritability (h^2) (Falconer, 1981). From this it follows that characters closely related to fitness, such as litter size, tend to have low heritabilities, while characters less obviously related to fitness, such as tail length in mice (Falconer, 1954), have high heritabilities. Characters closely related to fitness should feature nonadditive genetic variance most prominently, exhibit depression upon inbreeding, and show heterosis when animals are outbred.

Three techniques are available to behavioral geneticists to explore the relationship of a given behavior to fitness. First, one could estimate the heritability of a particular behavior and, if h^2 is low, conclude that the behavior in question is probably related to fitness. Low heritabilities often indicate that environmental variances are large, and another approach to determining whether low heritabilities indicate a relation to fitness would be to conduct Matherian analyses to compare broad-sense and narrow-sense heritabilities from data obtained between two or more inbred strains and the appropriate analysis of backcross, F_1, and F_2 generations. Second, one could outbreed animals and, if the behavior shows heterosis, conclude that the phenotype is related to fitness. Third, one could inbreed animals systematically and, if particular behavioral phenotypes exhibit inbreeding depression, conclude that the traits are closely related to fitness. The first two techniques have been used frequently in behavioral genetic research, albeit too often to answer different genetic questions. To our knowledge, there are no experiments on the effects of inbreeding on the behavior of experimental animals.

There have been many studies of the effects of inbreeding on morphological and physiological traits, yet the consequences of a systematic program of inbreeding on behavioral traits apparently have not been explored (Roberts, 1967). For this reason, one of the purposes of the present experiments was empirical: that is, to determine if there are effects of inbreeding on certain measures of learning and locomotor behavior and, if so, to assess the magnitude of these effects. The second purpose was more theoretical: to determine whether any of the behaviors under investigation might be a component of fitness.

METHODS

Subjects

To begin inbreeding, 20 brother–sister pairs of mice were selected randomly at 59 days of age from a genetically heterogeneous stock of

mice (HS/lbg) maintained by the Institute for Behavioral Genetics, Boulder, Colo. The origin and breeding plan for these HS/Ibg mice have been described by McClearn *et al.* (1970). After completion of the behavioral test battery, the pairs were mated to produce the first inbred generation. When the litters were 59 days of age, a brother–sister pair from each litter was randomly selected, given the behavioral test battery, and mated to produce the second inbred generation. This procedure was maintained through six generations. At this point, the experiment had to be discontinued because too few matings were fertile. While subjects of the fifth inbred generation were being tested, 22 59-day-old HS/Ibg mice were randomly selected and tested to serve as an outbred comparison group.

Apparatus

The open-field apparatus consisted of a 91.5 × 91.5-cm gray Plexiglas floor, divided by black lines into 36 equal squares. This apparatus and the procedure used to measure locomotor activity have been described in detail by McClearn (1960).

The training apparatus for the active avoidance task consisted of a Plexiglas box 18 cm in height and 17 cm in width and length. A grid floor mounted 2.5 cm above the base consisted of 2.38-mm stainless-steel rods, separated by 6.35 mm measured between centers. Around the inside walls of the box was mounted a shelf 3.81 cm wide and 6.67 cm above the floor. A microswitch activated a buzzer (attached to the box) and a 100-W light bulb mounted 25.4 cm above the box. This apparatus and the procedure used to train and extinguish the animals have been described in detail by Schlesinger and Wimer (1967).

The apparatus used in the passive avoidance task was a Plexiglas box 17.8 cm high and 15.4 cm square. Extending from one side of this compartment was a narrow alley 20.3 cm long and 2.54 cm wide. This apparatus and the procedure used to train the animals have been described in detail by Boggan and Schlesinger (1974).

Procedure

All offspring were housed with their parents in standard metal cages until weaning at 21 days of age. After weaning and before testing, like-sexed littermates were housed together under standard conditions with food and water available *ad libitum*. After selection and during testing, subjects were housed individually. All testing was performed between 1 and 5 PM. For each generation, half the mice were tested according to Schedule A and half followed Schedule B.

Age (days)	Schedule A	Schedule B
59	Isolated	Isolated
60	Open field	Open field
61	Open field	Open field
62	Active avoidance	Passive avoidance
63	Rest	Passive avoidance
64	Passive avoidance	Rest
65	Passive avoidance	Active avoidance
66	Mating	Mating

Half of the males and half of the females were tested on each schedule. After completion of the behavioral tests, brother–sister pairs were mated and collection of reproductive data began. These data included (1) number of days to the birth of the first litter, measured over seven generations, (2) number of pups in the first litter, measured over seven generations, (3) number of pups surviving to weaning, measured over seven generations, and (4) weight of pups at weaning, measured over five generations.

Behavioral Tests

Open Field

On the first day of testing, each mouse was placed in the starting corner for 60 s and then released. A record was kept of (1) latency to move out of the starting square, (2) number of squares entered in the first 5-min and second 5-min periods of testing, (3) number of center squares (away from the walls) entered in the first and second 5-min period, and (4) number of fecal boli dropped during the first and second 5-min period. The same procedures were followed on the second day of activity measurement.

Active Avoidance

Initially, each animal was placed on the inside shelf for 30 s and then on the grid floor for 30 s. At the end of this short adaptation period, the conditioned stumulus (CS; a buzzer and a light) was presented for 3 s. The cessation of the CS was coincidental with the onset of the unconditioned stimulus (UCS; a 0.2-mA shock at 340 V dc to the animal's feet delivered through the grid floor) and the start of an automatic timing device. The shock and the automatic timing device stayed on until the animal jumped to the shelf. The animal remained on the shelf during the intertrial interval of 15 s. The mouse was then placed on the grid floor

and the next trial began. Training continued until the mouse reached the learning criterion of 8 avoidance responses in 10 trials. An avoidance response was defined as jumping onto the shelf during the CS interval. If the animal did not reach the acquisition criterion within 50 trials, training was discontinued and the mouse was judged not to have learned the response. Those animals that reached the acquisition criterion were then given a series of extinction trials on which the shock device was disconnected and only the CS occurred. Extinction trials continued until the animals reached the extinction criterion of remaining on the grid floor 8 of 10 times for 30 s after the onset of the CS.

Passive Avoidance

On the first day each animal was placed at the end of the alley most distant from the large square enclosure. The latency for the animal to move from the starting point into the enclosure was measured. When all four paws were placed inside the enclosure, the animal was immediately given a 1-mA at 340 V dc foot shock delivered through the grid floor. It was then returned to its home cage. Twenty-four hours later each mouse was again placed at the end of the alley, and the time that elapsed before it entered the enclosure was recorded. If the animal failed to reenter the enclosure within 3 min, it was removed from the alley and given a score of 180 s. The measure of retention that was used was trial 2 minus trial 1 latency.

RESULTS

As shown in Fig. 1, there was a decrease in several measures of reproductive behavior that is indicative of a decrease in fitness as a function of inbreeding. A major effect was a decrease in the number of fertile matings. Originally, all 20 pairs of matings produced offspring that survived to weaning. By the fifth generation of inbreeding ($F = .67$), only 13 mating pairs were available; of these, only 10 produced offspring. In the sixth generation, only nine mating pairs were available and only five produced offspring that survived to weaning. By the seventh generation, only three mating pairs were available and only two of these produced offspring. Because of this dramatic decrease, it was deemed futile to continue the experiment.

The decrease in the size of the first litter was not statistically significant [$F(6,95) = 1.16$]. The number of pups weaned per litter decreased significantly over the course of inbreeding [$F(6,95) = 5.75$, $p < .01$]. The decrease in the weight of the pups also was significant [$F(6,63) = 5.86$, $p < .01$], as was the increase in the amount of time to deliver the first

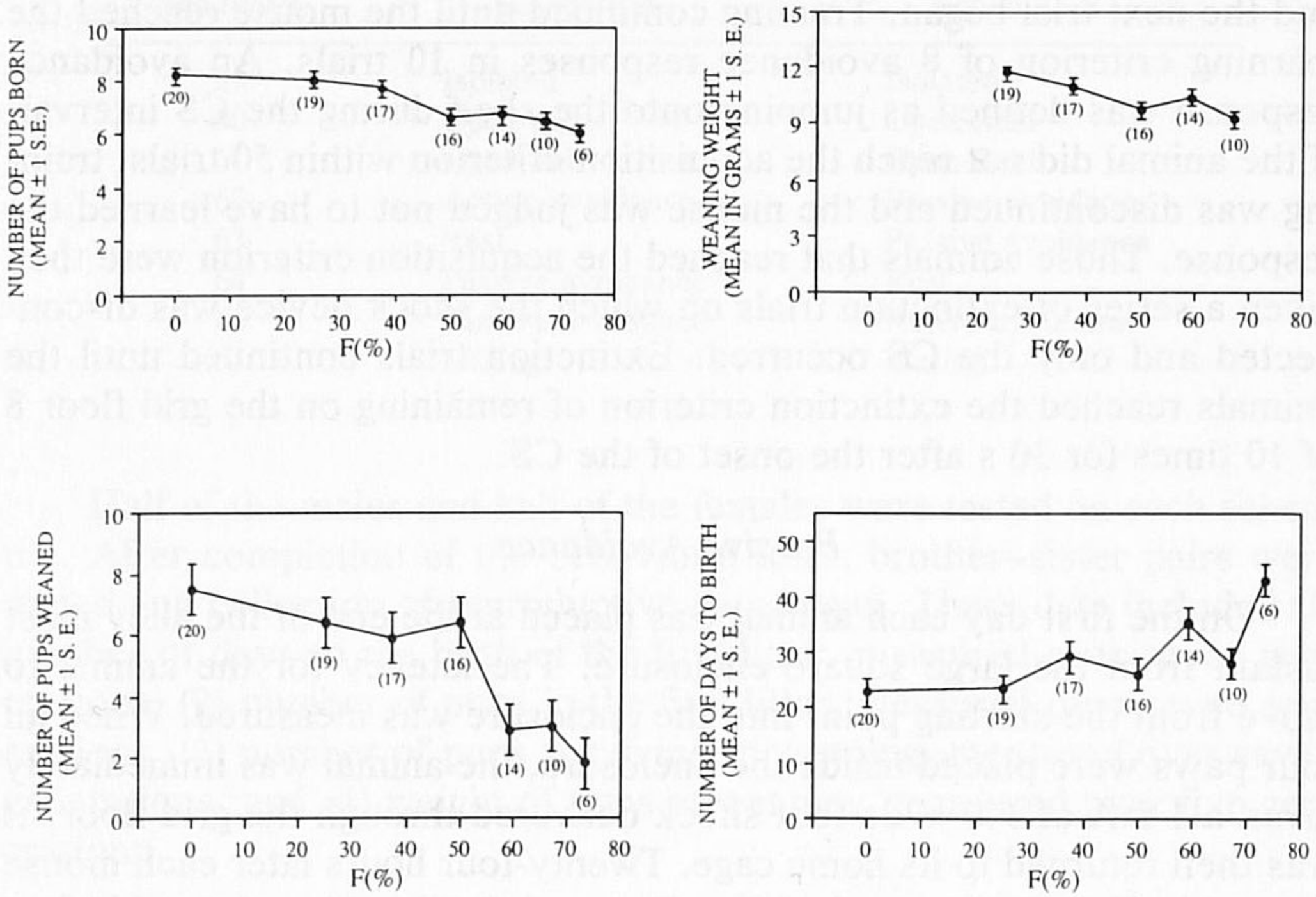

Fig. 1. Measures of reproductive fitness. Data are plotted as a function of degree of inbreeding. Averages ± 1 SE are plotted; numbers in parentheses = *N*.

litter after the animals were placed together [$F(6,95) = 3.58$, $p < .01$]. These data are summarized in Fig. 1.

As shown in Fig. 2, there were several changes in active avoidance learning as a function of inbreeding. First, there was a significant increase in the number of trials required to reach the acquisition criterion [$F(1,96) = 12.48$, $p < .01$]. Furthermore, it is of interest to note not only that the number of trials to reach criterion increased, but also that the percentage of animals that never learned the response at all increased from 2.5% in the HS population to 41.6% in the fifth inbred generation. With respect to extinction, there was a steady decline in the average number of trials required to reach the extinction criterion [$F(1,71) = 5.74$, $p < .01$]. In general, these data indicate that these animals took more trials to learn the response and fewer trials to extinguish the response as inbreeding progressed.

The performance of the animals on the passive avoidance task was also affected by level of inbreeding. Although the retention scores did not increase monotonically as a function of inbreeding, trial 2 minus trial 1 latency measures increased from 15.1 s in the HS animals to 71.8 s in the fifth inbred generation. The overall increase in trial 2 minus trial 1 latency

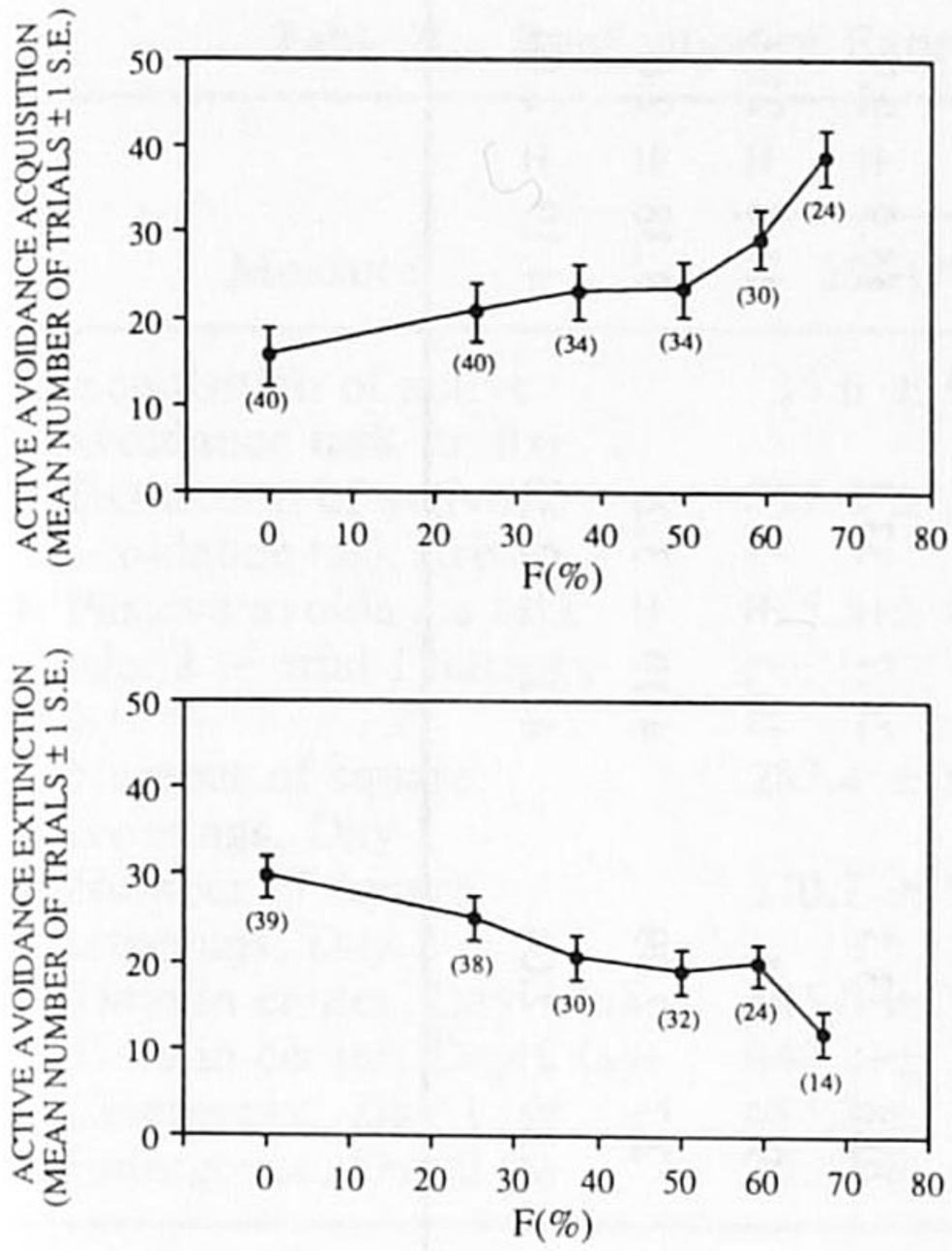

Fig. 2. Measures of learning. Data are plotted as a function of degree of inbreeding. Averages ± 1 SE are plotted; numbers in parentheses = *N*.

scores was statistically significant [$F(1,98) = 4.73$, $p < .01$]. Too few animals were available for analysis in the sixth generation.

The data on the various measures of locomotor activity are summarized in Table I. Overall, measures of locomotor activity showed little change as a function of inbreeding. Only two measures showed a reliable change: total number of square crossings on day 2 [$F(1,98) = 2.65$, $p < .05$] and emergence on day 1 [$F(1,98) = 2.60$, $p < .05$]. Analysis of difference scores between day 1 and day 2 of testing revealed only one reliable difference: number of squares crossed in the first 5 min on day 1 minus second 5-min score on the same day [$F(1,98) = 3.82$, $p < .01$].

Behavioral measures taken on the comparison group of HS animals (measurements taken at the same time the fifth generation of inbred animals were being tested) were not significantly different from those of the

Table I. Effects of Inbreeding on Measures of Locomotor Activity[a]

Measure	Generations of brother × sister mating					
	0	1	2	3	4	5
1. Number of square crossings, Day 1	287.4 ± 90.8	351.2 ± 174.8	332.7 ± 115.8	337.0 ± 141.6	345.0 ± 145.4	399.4 ± 170.3
2. Number of square crossings, Day 2	270.7 ± 90.9	350.0 ± 180.2	331.6 ± 124.4	337.2 ± 143.5	356.6 ± 149.1	396.7 ± 184.9
3. Time in center, Day 1 (s)	45.8 ± 36.4	37.6 ± 28.6	40.1 ± 28.1	33.2 ± 32.7	56.1 ± 42.2	41.2 ± 25.8
4. Time in center, Day 2 (s)	40.2 ± 28.2	28.2 ± 19.2	42.0 ± 42.2	35.1 ± 28.8	42.7 ± 38.5	41.5 ± 27.4
5. Emergence, Day 1 (s)	13.3 ± 11.7	40.8 ± 62.8	25.6 ± 36.8	20.8 ± 33.3	24.7 ± 33.3	15.9 ± 9.7
6. Emergence, Day 2 (s)	5.42 ± 4.19	16.12 ± 31.1	11.9 ± 12.4	9.8 ± 8.5	10.1 ± 11.5	11.8 ± 21.6
7. Number of boli, Day 1	3.95 ± 2.3	4.0 ± 2.07	3.97 ± 2.5	2.7 ± 2.09	4.10 ± 3.2	3.38 ± 2.0
8. Number of boli, Day 2	40.7 ± 2.8	4.58 ± 2.41	4.85 ± 2.41	3.35 ± 2.04	4.17 ± 2.35	4.19 ± 2.04

[a] Measures are given as the population mean ± 1 SD.

Table II. Replication of Experiment on HS/Ibg Stock Populations

Measure	$X \pm$ SD				
	HS-O[a]	HS-control[b]	t	df	p
1. Acquisition of active avoidance task (trials)	15.6 ± 9.1	16.6 ± 4.1	.32	48	NS
2. Extinction of active avoidance task (trials)	29.7 ± 15.5	32.6 ± 15.5	.52	47	NS
3. Passive avoidance task, trial 2 − trial 1 laltency (s)	15.1 ± 42.2	17.9 ± 35.4	.26	60	NS
4. Number of square crossings, Day 1	287.4 ± 90.8	317.5 ± 88.4	1.27	60	NS
5. Number of square crossings, Day 2	270.7 ± 90.9	299.4 ± 96.9	1.16	60	NS
6. Time in center, Day 1 (s)	45.8 ± 36.4	50.6 ± 29.5	.54	60	NS
7. Time in center, Day 2 (s)	40.2 ± 28.2	37.6 ± 15.1	.39	60	NS
8. Emergence, Day 1 (s)	13.3 ± 11.7	14.5 ± 13.7	.39	60	NS
9. Emergence, Day 2 (s)	5.42 ± 4.19	8.9 ± 5.5	2.75	60	<.01

[a] Original HS population from which animals were inbred.
[b] HS population tested at the same time the fifth inbred population was tested.

HS base population, with one exception: open-field emergence time on day 2 of testing was different in the two HS samples [$t(60) = 2.75$, $p < .01$]. These data are summarized in Table II.

DISCUSSION

Inbreeding produced a marked decline in the reproductive capacity of the population. The decline is similar to that which would be predicted if the foundation population had been wild and randomly mating. What is remarkable about the decrease in reproductive ability in these particular lines is that the foundation population, the HS/Ibg animals, were produced by crossing inbred strains in which there are no lethal genes. In the absence of such lethal genes in the gene pool of the foundation population, these mice still suffered inbreeding depression in most of the measures of reproduction we used. It seems pertinent at this point to discuss the advisability of using these HS animals in this kind of study. The HS mice by definition have an inbreeding coefficient equal to 0. In fact, however, these mice are inbred to a small degree. It may make more sense, in terms of extrapolating to natural populations, to use these partially inbred animals, rather than truly outbred animals, in these experiments, because mice living in demes in the wild are known to inbreed substantially (Selander, 1970).